Gideon Hirschfield
David Adams
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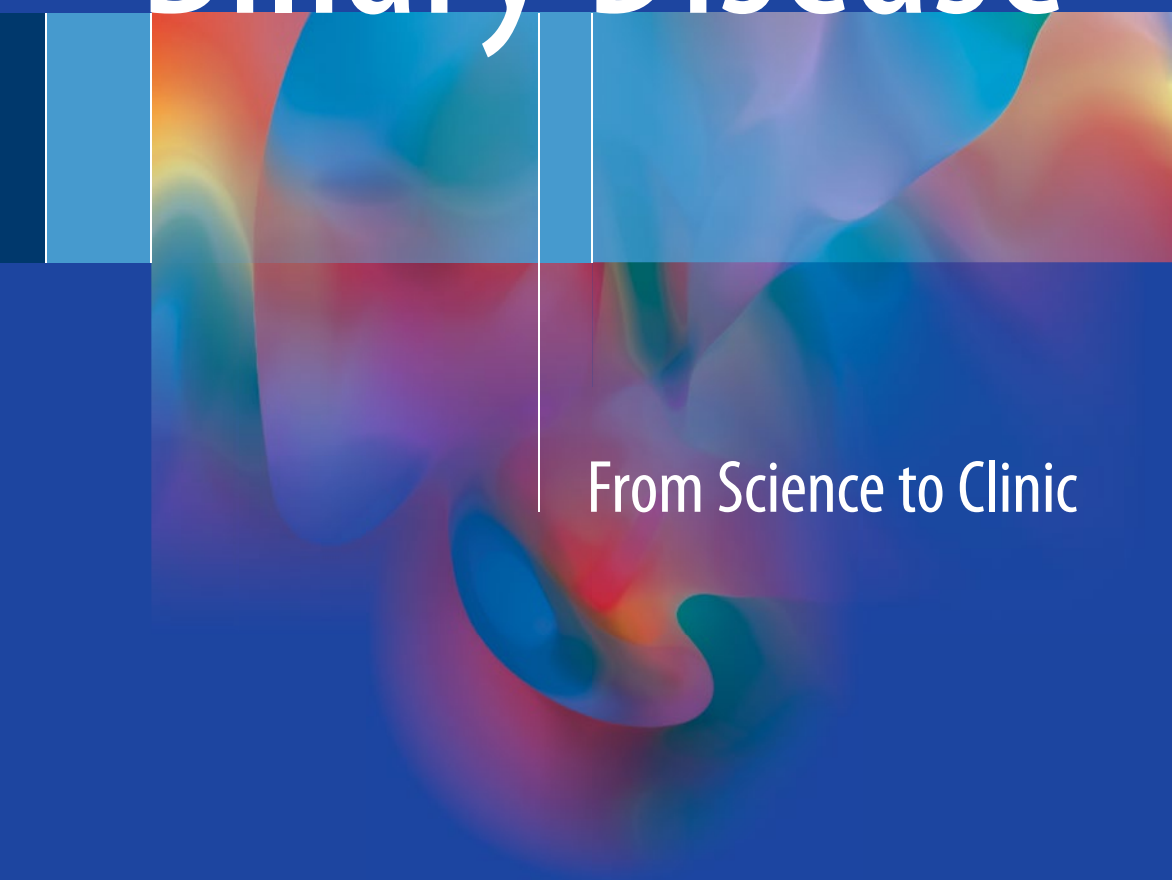
Editors

Biliary Disease



From Science to Clinic

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ISBN 978-3-319-50166-6 ISBN 978-3-319-50168-0 (eBook)
DOI 10.1007/978-3-319-50168-0

Library of Congress Control Number: 2017933709

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Biliary diseases are important causes of acute and chronic liver illnesses that span all ages, both sexes, and individuals wherever they reside. Whilst our treatments remain presently limited, the biologic understanding of disease continues to improve. With insights into all facets of biliary disease from epidemiology, through genetics and environmental risk, to local tissue responses, a more detailed pathway can be mapped that helps patients and clinicians understand the challenges they encounter. In some cases diseases are restricted by age, or biliary location, in other facets disease features overlap (immune pathways, impact of the microbiome, resultant symptoms), and such similarities and distinctions have advanced therapeutic targets, such that treatment beyond the non-specific bile acid, ursodeoxycholic acid, is reaching the clinic environment. These continue to include efforts to redirect immune responses against the biliary tree, drugs positioned to enhance protective gut-liver axes (FGF19-FXR/PPAR signalling) as well as agents that complement inherent biliary epithelial cell tolerability to injury by improved bicarbonate production in the biliary tree. This short book provides the reader a thorough journey through biliary disease and will act as a current primer to what is a dynamic and exciting area of liver disease.

Birmingham, UK

Gideon Hirschfield
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The Clinical Burden of Biliary Disease: A Global Perspective

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Kirsten Muri Boberg

Abstract

Many biliary disorders are considered rare diseases (diseases affecting less than 50 per 100,000 inhabitants) according to the definition by the European Commission for Public Health. They still impose a burden on the patients affected and the health-care system. Although adequate population-based epidemiological studies on biliary diseases are scarce from several parts of the world, it is evident that there is a marked geographical variation in the incidence and prevalence rates of many of the conditions (Table 1.1). These observations are attributed to differences in the worldwide distribution of risk factors. Biliary diseases may be diagnosed in all age groups, even in the neonate. The disorders range from benign conditions with potential curative options, including liver transplantation, to devastating biliary tract cancers with very poor survival. Hopefully, ongoing efforts to elucidate the pathogenesis and define potential targets for therapy for biliary disorders will reduce the burden of these conditions in the future.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_1

Take-Home Points

- Gallstone disease is one of the most common digestive disorders, with a prevalence of gallstones in Europe and the USA in the range of 6–22% and even higher numbers in some ethnic subgroups like American Indians.
- Extrahepatic biliary atresia is the most frequent cause of morbidity of all childhood hepatobiliary diseases and the most common indication for liver transplantation in children.
- There is large variation in the incidence and prevalence rates of the chronic cholestatic disorders primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) worldwide, with the highest occurrence reported from Northern Europe and North America.
- Gallbladder cancer is the most common malignancy of the biliary tree and among the five to six most common cancers of the gastrointestinal tract. High-risk populations are found in Latin America and Asia, while incidence rates are lower in Northern Europe and the USA.
- The incidence of intrahepatic cholangiocarcinoma varies widely worldwide, most likely associated with the variable distribution of risk factors. Asians are affected almost twice as frequently as whites and blacks, with the highest incidence rates found in Northeast Thailand. Both cholangiocarcinoma and gallbladder cancers carry a poor prognosis with 5-year survival around 10%.

1.1 Pediatric Cholestatic Disorders

Cholestasis in children can be a manifestation of a number of disorders [1]. Some of the pediatric cholestatic conditions affect the neonate, and it is important to differentiate these from a benign, transient neonatal jaundice. Several of the cholestatic syndromes that occur in infancy can result in significant childhood disease. Early awareness facilitates relevant diagnostic workup and subsequent therapy for the conditions where this is a possibility. A detailed history, including the time of onset of jaundice, may be helpful in the differential diagnosis. The causes of infantile cholestatic syndromes can be classified as extrahepatic or intrahepatic disorders [1].

1.2 Extrahepatic Biliary Atresia

Extrahepatic biliary atresia, consisting of obliteration of the hepatic or common bile duct, is the most frequent cause of morbidity of all childhood hepatobiliary diseases [1]. Early diagnosis is essential so that surgical treatment by portoenterostomy (Kasai procedure) can be carried out in due time to achieve the best possible result. Despite this surgical intervention, 2/3 of patients experience progressive liver disease with development of cirrhosis. Extrahepatic biliary atresia is the most common indication for liver transplantation in childhood [1].

The incidence of biliary atresia varies between geographical regions and appears to be higher in Asian countries than in Europe. The annual incidence per 10,000 live births has been reported to be 1.03–1.08 in Japan, 1.06 in Hawaii, 0.65–0.85 in the USA, 0.71 in Sweden, 0.70 in Australia, 0.48–0.59 in the UK, 0.51 in metropolitan France, and 0.50 in the Netherlands [2] (Table 1.1). A very high incidence of 3.2 per 10,000 live births has been observed in French Polynesia, followed by 1.32–1.65 per 10,000 in Taiwan [2]. Girls are more frequently affected than boys.

The pathology and treatment of biliary atresia is discussed in Chapter 6.

1.3 Progressive Familial Intrahepatic Cholestatic Syndromes

Progressive familial intrahepatic cholestasis (PFIC) comprises a heterogenous group of autosomal recessive disorders that are characterized by intrahepatic cholestasis. The conditions display characteristic clinical, biochemical, and histologic features. Genetic studies have revealed mutations in genes encoding hepatocanalicular transport proteins that are required for normal canalicular bile flow [3]. Mutations in the genes *ATP8B1*, *ABCB11*, *and ABCB4* are associated with the clinical entities PFIC syndromes type 1, 2, and 3, respectively [4]. Patients often present with cholestasis in the neonatal period or the first year of life, but PFIC3 may become apparent later in childhood or even during young adulthood [5]. Most PFIC patients will become liver transplant candidates.

Mutations in the *ATP8B1* gene can also cause benign recurrent intrahepatic cholestasis (BRIC1), characterized by episodic cholestasis at any age. A proportion of patients suffering from intrahepatic cholestasis of pregnancy have mutations in the *ABCB4* gene [3].

PFIC is a rare disorder, with an estimated incidence between 1 per 50,000 and 1 per 100,000 births, although exact numbers are not known [5]. PFIC appears to affect girls and boys equally frequent. All PFIC types have a worldwide distribution.

1.4 Drug-Induced Cholestasis

Drugs and other substances, including herbs and dietary supplements, may cause a wide range of liver damage. It is therefore important to consider drug-induced liver injury (DILI) as a differential diagnosis in all cases of hepatobiliary disease with uncertain etiology. DILI is usually classified as being of a hepatocellular, cholestatic, or mixed type, and the various drugs are usually associated with a predominating type of reaction. Genetic variants of biliary transporters (e.g., MDR3 and BSEP) have been associated with a predisposition to drug-induced cholestasis [3]. In most cases, the cholestatic drug-induced liver injury is mild and reversible, but persistent injury with biliary fibrosis and cirrhosis develops in some cases [6, 7].

Table 1.1 Examples of reported incidence and prevalence rates of biliary diseases

Disorder	Gender distribution	Incidence rates	Prevalence rates
Extrahepatic biliary atresia	Girls > boys	Per 10,000 live births: French Polynesia 3.2 Taiwan 1.32–1.65 Japan 1.03–1.08 USA 0.65–0.85 Sweden 0.71 Australia 0.70 UK 0.48–0.59 France 0.51 The Netherlands 0.50	n.a.
Progressive familial intrahepatic cholestasis	Girls ~ boys	1 per 50,000–1 per 100,000 live births	n.a.
Drug-induced cholestasis		Per 100,000 inhabitants/year: France: 8.1 (47% cholestatic plus mixed type) Spain: 3.4 (20% cholestatic, 22% mixed type) Iceland: 19.1 (32% cholestatic, 26% mixed type)	n.a.
Primary biliary cholangitis	Females:males ~ 10:1	Per 100,000 inhabitants/year: UK (Newcastle upon Tyne) 5.8 USA (Olmsted County) 2.7 Iceland 3.4 Finland 1.7 Norway 1.6 Sweden 1.3–2.4 The Netherlands 1.1	Per 100,000 inhabitants: UK (Newcastle upon Tyne) 39.2 USA (Olmsted County) 40.2 Iceland 38.3 Finland 18.0 Norway 14.6 Sweden 9.6–15.1 The Netherlands 13.2 Japan 2.7–5.4 Israel 5.5 Australia 1.91

Primary sclerosing cholangitis	Females/males ~ 1:2	Per 100,000 inhabitants/year: UK 0.41–0.91 USA (Minnesota) 0.90 Canada 0.92 Norway 1.31 Sweden 1.22 The Netherlands 0.5 Southern Europe and Asia <0.1	Per 100,000 inhabitants: Northern Europe and the USA 8–16 The Netherlands 6.0 Spain 0.22
Autoimmune pancreatitis (no specific data are available on IgG4-associated cholangitis)	Females<males	Per 100,000 inhabitants/year: Japan 0.9	Per 100,000 inhabitants: Japan 2.2
Intrahepatic cholangiocarcinoma	Males 1.3–3.3 × females	Per 100,000 inhabitants/year USA (1995–1999) 0.85 USA (2004–2007) 0.89 England and Wales (1995–2008) 1.62	n.a.
Extrahepatic cholangiocarcinoma		Per 100,000 inhabitants/year USA (1998) 0.82 USA (2004–2007) 0.99 England and Wales (1995–2008) 0.47	n.a.
Gallbladder cancer	Females 2–6 × males	Per 100,000 inhabitants/year India (Dehli, females) 21.5 Pakistan (South Karachi) 13.8 Ecuador (Quito) 12.9 Northern Europe, USA, Canada <3 (females)	n.a.
Gallstone disease	Females>males	n.a.	Per 100 inhabitants: USA (gallstones or previous cholecystectomy) 14.3 North American Indians 60–70 Europe 6–22 South America 9–29 Black Africans <5 Asia 3–6

Abbreviations: *USA* United States of America, *UK* United Kingdom, *n.a.* not applicable

There is only limited information on the incidence of DILI. In general, the frequency of drug-induced adverse events appears to be underestimated due to incomplete ascertainment of cases. In large DILI series, a cholestatic type of drug injury has been noted in 20–40% of patients [6]. In a population-based study from France from 1997 to 2000, the global crude annual incidence rate for hepatic adverse drug reactions was 13.9 ± 2.4 per 100,000 inhabitants, with a corresponding standardized annual global rate of 8.1 ± 1.5 [8]. For comparison, the annual detection rate of hepatitis C in the same region was approximately 30 per 100,000. Among the 34 cases of DILI recorded, cholestatic and mixed injury patterns accounted for 16 (47%). In a prospective study in Southern Spain during 1994–2004, 461 cases of toxic liver injury were collected. The estimated annual incidence rate in the period 1998–2003 (only reported from the coordinating center) was 3.4 ± 1.1 cases per 100,000 inhabitants, about half of these being classified as serious adverse hepatic reactions [9]. Among the 461 cases, a hepatocellular damage pattern was most common (58%), whereas the mixed and cholestatic types were equally frequent (22% and 20%, respectively). Patients with a cholestatic pattern were significantly older than patients with other patterns of liver injury, as has also been observed by others [7]. A prospective, population-based survey from Iceland during 2010–2011 reported a crude annual incidence rate of DILI as high as 19.1 (95% CI 15.4–23.3) cases per 100,000 inhabitants (excluding cases with acetaminophen toxicity) [10]. Hepatocellular injury was most frequent (42%), followed by the cholestatic (32%) and mixed types (26%). In a recent study from the DILIN Prospective Study in the USA, including 899 cases considered as DILI enrolled between 2004 and 2013, the pattern of damage was hepatocellular in 54% and cholestatic or mixed in 23% each [7]. Among the 899 patients, 10% died or underwent liver transplantation, underscoring the potential serious outcome of DILI. As many as 31% of patients with cholestatic injury had signs of chronic or unresolved injury 6 months after onset, compared with an overall frequency of 18%.

The above studies were carried out in Western countries; even less information on the occurrence of DILI is available from other parts of the world. The real impact of cholestatic DILI on the clinical burden of cholestatic liver disease is thus difficult to assess.

Chapter 7 focuses on the mechanisms and importance of drug-induced cholestasis.

1.5 Primary Biliary Cholangitis

Primary biliary cholangitis (PBC), previously known as primary biliary cirrhosis, is a chronic, non-suppurative, destructive cholangitis involving the small- to medium-sized intralobular bile ducts [11]. The disease leads to fibrosis and may progress to liver cirrhosis and end-stage liver disease. The diagnosis is conventionally based on a history of elevated cholestatic liver tests for at least 6 months, the presence of antimitochondrial antibodies, and a compatible liver biopsy [12]. According to current guidelines, a liver biopsy is not necessary to diagnose PBC [12]. This disorder predominantly affects females, with a female to male ratio of approximately 10:1. The median age of diagnosis is around 50 years; PBC does not affect children.

The pathogenesis of PBC appears to comprise a complex interaction between genetic and environmental factors, also involving autoimmunity. Ursodeoxycholic acid (UDCA) improves liver tests and delays the disease progression with extended liver transplant-free survival [13]. Around 40% of patients do not, however, display a biochemical response to UDCA. Hepatocellular carcinoma is a rare complication of PBC but occurred more frequently among nonresponders than responders to UDCA in a recent survey that included a large international cohort of 4565 PBC patients [14]. The overall incidence rate of hepatocellular carcinoma was 3.4 cases per 1000 patient-years. PBC patients who develop end-stage liver disease are good candidates for liver transplantation.

There is a large variation in the incidence and prevalence rates of PBC worldwide, ranging from 0.33–5.8 per 100,000 inhabitants/year and 1.91–40.2 per 100,000 inhabitants, respectively [15]. The highest incidence and prevalence rates have been reported from Northern Europe and North America, in particular including the findings in Newcastle upon Tyne, UK (incidence 5.8 per 100,000; prevalence 39.2 per 100,000) [16], and Olmsted County, USA (incidence 2.7 per 100,000; prevalence 40.2 per 100,000) [17]. Incidence rates per 100,000 for PBC from other northern European countries include 1.7 in Finland, 1.6 in Norway, 1.3–2.4 in Sweden, and 3.4 in Iceland [15, 18]. Corresponding prevalence figures per 100,000 inhabitants are 18.0 in Finland, 14.6 in Norway, 9.6–15.1 in Sweden, and 38.3 in Iceland. The largest population-based study on the epidemiology of PBC published until now was performed in the Netherlands during 2000–2008, comprising a population of around 5.8 million [18]. The mean annual incidence was 1.1 per 100,000, and the point prevalence at study end was 13.2 per 100,000. Incidence and prevalence rates increased significantly over time. Apparent increases in incidence and prevalence rates for PBC have also been noted by others, although several contributing factors may be implicated [19].

Good epidemiological studies on PBC from other parts of the world are scarce, but reports indicate generally lower incidence and prevalence numbers. Examples of published prevalence rates per 100,000 include studies from Japan (2.7–5.4), Israel (5.5), Australia (1.91), and Brunei Darussalam (Southeast Asia) (2.6) [15, 19].

Chapter 8 elaborates on the science and practice of PBC.

1.6 Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is characterized by inflammatory and fibrotic processes that primarily affect the medium-sized and large bile ducts, resulting in bile duct irregularities with strictures and dilatations [20]. PSC is more common in males than in females (2:1). The disease affects a rather young population, with median age at diagnosis around 40 years. PSC may also present in childhood and then often with associated features of autoimmune hepatitis [21]. PSC is in most cases a progressive disorder that leads to liver cirrhosis, with estimated survival from diagnosis until PSC-related death or liver transplantation in the range 13–21 years [22]. There is no medical therapy that effectively halts the disease progression, but PSC patients are considered good candidates for liver transplantation. In the Nordic countries, PSC has until recently been the most important indication

for liver transplantation [20]. PSC is strongly associated with inflammatory bowel disease (IBD). The highest frequencies of concomitant IBD (70–80%) have been reported from Northern Europe and the lowest (20–50%) from Asia [23].

PSC patients carry an increased risk of cancer. The high risk of cholangiocarcinoma (CCA) development (6–13% in population-based series) in particular adds to the disease burden in PSC patients. In a Swedish cohort of PSC patients, the risk of hepatobiliary malignancy was 161 times higher compared to the general Swedish population and that of cancer of the colon-rectum was ten times increased [24]. The risk of any gastrointestinal cancer was 29 times higher than in the general population. It is recognized that the risk of colorectal malignancies is higher in PSC-IBD than in IBD without hepatobiliary disease.

Epidemiological studies of PSC are hampered by several factors. First, PSC has an insidious onset and can have a prolonged preclinical course before being diagnosed. Approximately 50% of patients are asymptomatic at the time of diagnosis. Second, the diagnosis relies on typical findings by cholangiography and requires that such investigations have been carried out. Incidence and prevalence estimates therefore must be considered minimum numbers. Based on six population-based studies from North America and Europe, the combined incidence rate of PSC was 1.0 (0.82–1.17) per 100,000 [25]. The prevalence of PSC in these regions is around 10 per 100,000 [20]. More specifically, studies have shown an incidence rate per 100,000 of 1.31 in Norway, 1.22 in Sweden, 0.92 in Canada, from 0.41 to 0.91 in the UK, and 0.90 in Minnesota, USA [26]. A lower incidence rate of 0.5 per 100,000 was however noted in a recent study from the Netherlands, based on the identification of 590 PSC patients in an area of almost eight million inhabitants [22]. Markedly lower incidence rates have been observed in Southern Europe (0.07 per 100,000 in Spain) and Asia [20]. An increasing trend in the incidence of PSC has been noted [15]. The prevalence ranges from 8 to 16 per 100,000 inhabitants in Northern Europe and the USA (6.0 in the above study from the Netherlands), while reported numbers are lower in Southern Europe (0.22 per 100,000 in Spain) and Asia.

Small-duct PSC is characterized by the same presenting features as the classical (large-duct) PSC, but displays a normal cholangiogram. The proportion of small-duct PSC relative to classical PSC has been reported to be in the range 5.8–16.3% [26]. Small-duct PSC appears to have a more benign course than classical PSC.

Increasing efforts are directed toward the understanding of disease pathogenesis in PSC, with the ultimate aim to define targets for therapy. Chapter 9 describes current concepts in PSC biology and strategies for new therapy.

1.7 Autoimmune Sclerosing Cholangitis/IgG4 Cholangiopathy

Autoimmune sclerosing cholangitis/IgG4 cholangiopathy, also designated IgG4-associated cholangitis (IAC), is the biliary manifestation of immunoglobulin G4-related disease which is characterized by inflammatory lesions and can involve a number of organs [27]. IAC is frequently associated with IgG4 disease of the

pancreas (autoimmune pancreatitis). The diagnosis of IAC and autoimmune pancreatitis is based on the HISORt criteria (a combination of histology, imaging, serology, other organ involvement, and response to therapy) [28]. The histological findings in IAC are characterized by dense lymphoplasmacytic infiltrates (>10 IgG4-positive cells per high-power field), storiform fibrosis, and obliterative phlebitis. IAC is also associated with elevated serum IgG4 levels, but levels may be normal in up to 20% of patients [28]. IAC presents with features similar to those of PSC, and the two conditions may be difficult to differentiate [29]. IAC may also be difficult to differentiate from malignant conditions of the pancreas and biliary tract. Elevated serum IgG4 levels were present in 9% among 127 PSC patients [30], and 15.6% of 122 PSC liver explants showed marked hilar IgG4 lymphoplasmacytic infiltration [31].

Age at presentation of IAC varies, but these patients are generally older at diagnosis than patients with classic PSC. In similarity with PSC, there is a male predominance in IAC, whereas concomitant IBD is more uncommon. IAC typically responds to corticosteroid therapy [29] and is therefore important to recognize.

Data on incidence and prevalence of IgG4-related disease are limited. Most reports on IAC and autoimmune pancreatitis have been published from Japan and the Far East. In a nationwide epidemiological survey of autoimmune pancreatitis in Japan in 2007, the estimated annual incidence rate was 0.9 per 100,000 inhabitants, with an overall prevalence rate of 2.2 per 100,000 [32]. With more clinical focus on these conditions, they are increasingly being recognized also in the Western world. Among 3482 cases of IgG4-related disease reported up to March 2014, 2621 originated from Asia, 470 from Europe, and 452 from North America [33]. No separate epidemiological data on IAC are available as of yet.

The IgG4-related biliary disease is further described in Chapter 12.

1.8 Cholangiocarcinoma

CCAs are classified as (1) intrahepatic CCA, (2) perihilar (Klatskin tumor) CCA, and distal CCA, the two latter collectively being defined as extrahepatic CCA. The majority (60–70%) of tumors are hilar, 20–30% are distal extrahepatic, whereas intrahepatic CCA accounts for 5–10% of cases [34, 35]. Intrahepatic and extrahepatic CCA differ regarding epidemiology, potential risk factors, clinical features, therapeutic options, and prognosis. CCAs constitute about 3% of all gastrointestinal cancers. Intrahepatic CCA is the second most common primary liver cancer after hepatocellular carcinoma and accounts for approximately 10% of primary liver cancers [36]. Hepatobiliary malignancies cause around 13% of cancer-related deaths globally, and 10–20% among these are due to CCA [37]. Most CCAs are sporadic, but a proportion (<30%) of cases develops on the background of established risk factors [35, 38]. Except for tumors developing in patients with PSC, CCA is rare before the age of 40, and the peak age is in the seventh decade [36]. Men are affected from 1.3 to 3.3 times more than women.

CCA typically presents at a late stage when curative therapeutic options are limited, and less than 1/3 of patients are resectable at diagnosis. Overall, the 5-year survival rate for CCA patients has not improved over the last decades and is only about 10% [39]. Due to the dismal prognosis of this malignancy, mortality and incidence rates are similar [40].

Over the last decades, several studies have reported increasing incidence and mortality rates for intrahepatic CCA, along with stable or decreasing rates for extrahepatic CCA on an international basis [34, 35, 38, 41]. Changes in the International Classification of Diseases for Oncology (ICD-O) may have influenced these observations since coding misclassifications of hilar CCA as an intrahepatic rather than extrahepatic malignancy have been likely [34]. The adoption of new editions of ICD-O at different time points in different countries may also have contributed to varying incidence rates for intra- and extrahepatic CCA [34]. Although the separate trends for intra- and extrahepatic tumors should be interpreted with caution, there appears to be an overall rising incidence of CCA.

There is a wide variation in the incidence of intrahepatic CCA in different parts of the world, most likely associated with the variable distribution of risk factors. Asians are affected almost twice more frequently than whites and blacks [36]. Low incidence rates have been reported from Australia with 0.2 and 0.1 per 100,000 in men and women, respectively [36], whereas Northeast Thailand has the highest incidence rate of intrahepatic CCA worldwide, with numbers up to 113 per 100,000 person-years in men and 50 per 100,000 in women [37]. This high risk of CCA in Thailand is related to the high prevalence of hepatobiliary fluke infestations (*Opisthorchis viverrini* and *Clonorchis sinensis*) [35, 42].

From a population-based study in the USA using data from the cancer registries of the Surveillance, Epidemiology, and End Results (SEER) program, the age-adjusted incidence rate of intrahepatic CCA increased by 165% from 0.32 per 100,000 in 1975–1979 to 0.85 per 100,000 in 1995–1999, with most of the increase occurring after 1985 [43]. The incidence of extrahepatic CCA declined by 14% from 1.08 per 100,000 in 1979 to 0.82 per 100,000 in 1998 [36]. An updated review of CCA incidence rates between 1992 and 2007 in the USA supported the previously reported increase in incidence of intrahepatic CCA and a relatively stable incidence of extrahepatic CCA up to 2000. However, when the investigation was extended to 2007, the incidence of intrahepatic CCA remained overall stable with only slight fluctuations, whereas the incidence of extrahepatic tumors had increased after 2000 [44]. During 2004–2007, the age-adjusted incidence rate for intrahepatic and extrahepatic CCA was 0.89 (95% CI 0.87–0.91) and 0.99 (95% CI 0.96–1.01) per 100,000, respectively. Misclassification of hilar CCAs did not appear to significantly affect these trends. These findings differ from those in a study of CCA in England and Wales between 1990 and 2008, where age-standardized incidence rates rose for intrahepatic CCA and fell for extrahepatic CCA [34]. A marked increase of intrahepatic CCA (from 0.87 to 1.62 per 100,000) and a decrease of extrahepatic CCA (from 0.55 to 0.47 per 100,000) remained in a reanalysis after transferral of

Klatskin tumors from the intrahepatic to the extrahepatic group for the period 1995–2008. Similar trends have been observed in some countries (Japan, Italy, Germany), whereas a study from Denmark found a fall in incidence of both intrahepatic and extrahepatic CCA between 1978 and 2002, and a report from France concluded with stable incidences of both tumor groups [42, 45].

A number of molecular alterations involved in the pathogenesis of CCA have now been identified. These are potential biomarkers for early diagnosis as well as representing possible targets for therapy. Several studies on targeted therapies in CCA are currently ongoing [46], as discussed in Chapter 10.

1.9 Gallbladder Cancer

Gallbladder cancer is the most common malignancy of the biliary tree, accounting for 80–95% of cases in autopsy studies [47]. It is among the five to six most common cancers of the gastrointestinal tract [48]. Overall, gallbladder cancer accounts for less than 1% of all cancer deaths [49]. Symptoms and signs of gallbladder cancer are nonspecific, and the condition is usually diagnosed at an advanced clinical stage when only 10–30% of patients are candidates for potential radical cholecystectomy [48]. The prognosis is dismal, with 5-year survival rates of only 0–12% [39, 49]. Gallbladder carcinoma is discovered in 1–2% of patients who undergo cholecystectomy for anticipated benign disease [48]. Several risk factors have been associated with the development of gallbladder carcinoma, the most important being a history of gallstone disease [48, 50].

The risk of gallbladder cancer increases with age [47]. Among 10,301 patients with a diagnosis of gallbladder carcinoma in the SEER program in the USA during the years 1973–2002, the median age at diagnosis was 73 years [49]. During the last decade, there was a significant decrease in the incidence of gallbladder carcinoma in patients older than 50 years, along with a slight increase in the incidence among younger individuals. Gallbladder cancer affects women more commonly (two to six times) than men [47, 50]. In another report from the SEER registry, the incidence of gallbladder cancer declined by 42% from 1979 to 1997 and was then stable through 2004. The death rate decreased by 48% from 1979 to 2004. The mortality rate was 6.8 times higher in the age group ≥65 years compared with those being 45–64 years.

There is a wide variation in the worldwide incidence of gallbladder carcinoma, and the incidence also varies among ethnic groups within countries [47]. In a search of cancer registries worldwide, Randi et al. [50] identified two major groups of high-risk populations, in Latin America and Asia, respectively. The highest overall incidence rates had been reported for women in Delhi, India (21.5 per 100,000), followed by Karachi South, Pakistan (13.8 per 100,000), and Quito, Ecuador (12.9 per 100,000). High incidence rates were also noted in the Far East Asia (Korea and Japan) and in some countries in Eastern Europe (Slovakia, Poland, Czech Republic,

and Yugoslavia) and South America (Columbia). Gallbladder cancer has even been reported to be the main cause of death from cancer among women in some areas of South America [50]. Low incidences of gallbladder cancer (<3 per 100,000 women and 1.5 per 100,000 men) were identified in most cancer registries from Northern Europe and from the USA (SEER registry) and Canada. In the USA, the incidence rate is higher among Hispanic women in California and New Mexico than in any other ethnic groups [47]. Age-adjusted mortality rates are around 50% higher in African Americans than in whites [39].

1.10 Gallstone Disease

Gallstone disease is one of the most common digestive disorders and represents a major health burden in developed countries, regarding morbidity as well as costs [51]. It is the most common cause of biliary tract disorder in adults. Cross-sectional gallbladder ultrasonography surveys have contributed to the estimation of prevalence rates. Cholelithiasis is a complex disease in which genetic components as well as multiple individual and shared environmental factors contribute to the pathogenesis [52], and these factors are likely to influence on the varied distribution of gallbladder stones worldwide. Gallbladder stones are more common in European countries and North and South America than in Asia and Africa [51, 52]. Prevalence of gallstones in Europe and the USA is generally in the range 6–22%, however, with higher frequencies in some ethnic subgroups [51, 52]. Intermediate prevalences, in the range 3–15%, are seen in Asian populations and lower numbers in Africa. Cholesterol gallstones dominate in Europe and North and South America, whereas bilirubin gallstones are more common in Asia and Africa.

From a national population-based survey comprising more than 14,000 persons in the USA, the prevalence of gallstones or previous cholecystectomy was 14.3% [53]. The prevalence was higher in women (16.6%) than in men (7.9%). Age-standardized prevalence was higher in Mexican Americans (males 8.9%, females 26.7%) and non-Hispanic white (men 8.6%, women 16.6%) than in non-Hispanic blacks (men 5.3%, women 13.9%). Gallbladder disease was estimated to affect more that 20 million persons (6.3 million men and 14.2 million women) aged 20–74 years [53]. A high proportion (30.4% and 48.2% of men and women, respectively) of persons with gallbladder disease had undergone cholecystectomy. The highest prevalence occurs in North American Indians, being up to 73% in Pima Indians older than 30 years [51]. High prevalence is also seen in Indians in South America.

In a report on digestive and liver disease statistics in the USA, gallstone disease was the most common principal gastrointestinal inpatient diagnosis in the year 2000, amounting to 262,411 hospitalizations and a median inpatient charge of USD 11,584 [54]. In addition, there were an estimated 778,632 outpatient visits.

Gallstone disease is an important health problem that requires continuing attention, and the current scientific understanding and controversies are further discussed in Chapter 11.

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The Healthy Biliary Tree: Cellular and Immune Biology

2

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Abstract

The biliary tree is an arborizing system of intra- and extrahepatic conduits connecting the liver to the intestine. The biliary tree has a complex tridimensional structure, encompassing bile ducts of different sizes, morphologies, and functions. The most studied function of biliary epithelial cells (cholangiocytes) is to regulate the hydration and alkalinity of the primary bile secreted by hepatocytes. An increasing number of evidence highlight the ability of cholangiocyte to undergo changes in phenotype, proliferation, and secretory activity in response to liver damage. Cholangiocytes are involved in biliary innate immunity; altered

Financial Support Cariplo grant 2014–1099 to MC; Telethon grant GGP09189 to LF; NIH Grants RO1DK096096, RO1DK-079,005-07, DK034989 Silvio O. Conte Digestive Diseases Research Core Center, and projects FFC 24/2015, and Telethon grant GGP12133 to MS.

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G. Hirschfield et al. (eds.), *Biliary Disease*, DOI 10.1007/978-3-319-50168-0_2

biliary innate immunity plays a role in a number of biliary diseases, including genetic cholangiopathies, such as cystic fibrosis-related liver disease. In addition, cholangiocytes may behave as antigen-presenting cells and secrete immunoglobulins as well as several antimicrobial peptides. Thus, cholangiocytes, by participating actively to the immune and inflammatory responses, represent a first defense line against liver injury from different causes. In fact, cholangiocytes possess a number of sensing receptors for pathogen-associated molecular patterns (PAMPs), such as Toll-like receptors (TLRs), which modulate their proinflammatory behavior. Derangements of the signals controlling these mechanisms are at the basis of the pathogenesis of different cholangiopathies, often extending beyond the classically recognized immune-mediated (primary biliary cirrhosis, primary sclerosing cholangitis), as in cystic fibrosis liver disease.

Take-Home Points

- The main function of the biliary epithelium is the transport of bile to the gallbladder and intestine and modification of the primary bile secreted by hepatocytes.
- Cholangiocytes, the cells lining the biliary epithelium, are active players in innate immunity and inflammation.
- They are able to secrete IgA (sIgA) into bile and a variety of peptides with antimicrobial properties.
- Upon injury, reactive cholangiocytes are able to secrete a number of proinflammatory and profibrotic cytokines, chemokines, and growth factors.
- They express HLA class I molecules and HLA class II in inflammation, but not co-stimulatory molecules CD80 and CD86; thus, they are unlikely to play a direct role in the activation of T cells during hepatic inflammation.

2.1 The Normal Biliary Tree

The biliary tree is a complex tridimensional network of epithelial conduits laying into the hepatic parenchyma and extending outside the liver up to the Vater's papilla. The intrahepatic and extrahepatic sections of the biliary tree have different functions. Ductal structures are lined by biliary epithelial cells (BECs), also termed as cholangiocytes. Although representing less than 5% of the total liver cells, cholangiocytes are involved in several physiological functions as well as in many pathological responses. Within the liver, the intrahepatic bile ducts start from the Canals of Hering (CoH), the interface structure composed partly by cholangiocytes and partly by hepatocytes. CoH are localized at the periphery of the portal tract where they link the bile canaliculi, formed by two juxtaposed hepatocytes, to the smallest ramifications of the bile duct system, entirely lined by BECs (bile ductules). Then the bile ducts gradually enlarge through different levels of organization, including interlobular, septal, areal, and, finally, segmental ducts, the largest intrahepatic

biliary structure. Then segmental ducts coalesce in the two main hepatic ducts, which merge at the level of the hepatic hilum to form the common hepatic duct, and, after receiving the cystic duct, they form the common bile duct that connects the liver and the gallbladder to the intestine. The intra- and extrahepatic portions of the biliary tree have different embryologic origins and differ in terms of functions, pathologic processes, and oncologic aspects.

The biliary tree collects the primary bile produced by hepatocytes and modifies its composition by hydration and alkalinization and then delivers the bile to the intestine where it is essential for fat absorption. Besides bile transport/modifications, the biliary epithelium is intimately involved in liver inflammation and in the regenerative/reparative responses to liver injury. The interlobular bile ducts are a preferred target in auto- and alloimmune diseases, while the smallest ducts are mainly involved in liver repair as they proliferate and expand in close relationship with the activation of the stem cell compartment. A growing body of evidence indicates an important role of cholangiocytes in liver innate immunity.

2.2 Development of the Bile Duct Epithelium

Phenotypic diversity of intrahepatic and extrahepatic bile ducts reflects their different embryological origin. While the extrahepatic bile ducts originate from the caudal part of the ventral foregut [14, 106] from a $Hex^{-}/Pdx\text{-}1^{+}/Sox17^{+}$ primordial cell population [124], the intrahepatic system is derived from the hepatoblasts, bipotent parenchymal cells present in the fetal liver bud, characterized by a $Hex^{+}/Pdx\text{-}1^{-}/Sox17^{-}/AFP^{+}$ phenotype. From the 8th gestational week (GW) onward, hepatoblasts surrounding the mesenchyme of the nascent portal space start to differentiate toward a biliary phenotype, leading to a cell monolayer called ductal plate (DP) [114], expressing phenotypic markers of both hepatocellular (keratin 8 (K8) and K18) and biliary lineages (K19) [117]. From the 12th to the 16th GW, DP duplicates to form luminal structures where the hepatocyte markers are progressively downregulated, while other biliary markers, including K7, are neo-expressed [114, 117]. Residual non-duplicating DP is deleted by apoptosis [113], although a back-transdifferentiation of the DP remnants to periportal hepatocytes has been recently hypothesized [17]. In the meanwhile, biliary structures with a ductal configuration gradually migrate to be incorporated into the portal mesenchyme [30, 32]. Notably, intrahepatic bile duct maturation develops in strict conjunction with the formation of both the arterial vasculature and the peribiliary plexus to provide them with the blood nourishment. The fine synchronization of this maturation process depends upon the balanced expression of a range of angiogenic growth factors, mainly VEGF-A and the angiopoietins, Ang-1 and Ang-2, and of the corresponding receptors, VEGFR1, VEGFR2, and Tie-2, by both DP cells and endothelial cells [32, 116, 121]. The cell fate specification toward a biliary phenotype and the correct assembly of biliary cells in a ductal configuration are regulated by a complex and finely tuned interplay of growth factors, transcription factors, morphogens, and miRNAs. They include members of the transforming growth factor (TGF), hepatocyte nuclear

factor (HNF), and sex-determining region Y(SRY)-related HMG box transcription factor (SOX) families, as well as effectors of Notch and WNT signaling, derived from the surrounding microenvironment [61, 124].

2.2.1 Morphogens and Transcription Factors Involved in Biliary Lineage Specification and Bile Duct Formation

Studies on zebra fish [96] and mouse [69, 123] embryos further demonstrated the role of Notch signaling in biliary development. Initially, Jagged1 (Jag1) expressed by the nascent portal mesenchyme induces the transdifferentiation to the biliary phenotype of the Notch2-expressing hepatoblasts localized in close contact to the mesenchyme. Following this interaction, the hairy and enhancer of split-1 (Hes1), a downstream effector of the Notch receptor, is activated and binds to the nuclear transcription factor recombinant signal binding protein for immunoglobulin kappa J (RBP-Jk) to stimulate the activation of the cholangiocyte-specific transcription factors HNF1β and Sox9 [7, 36]. In this phase, expression of the neural cell adhesion molecule (NCAM) in conjunction with the antiapoptotic protein Bcl-2 is an early signature of the developing ductal plate [29]. These primordial ductal structures have an asymmetrical configuration ("transient asymmetry"), as they are formed on the portal side by cells with a biliary phenotype and on the parenchymal side by hepatoblasts [61]. In the following phase, the neo-formed DP cells start to express Jag1, which, in turn, induces the transdifferentiation of a second layer of hepatoblasts to DP cells, thus acquiring a symmetrical configuration [7, 88]. In their maturation to symmetrical ducts entirely lined by cells with a biliary phenotype, DP cells progressively loose NCAM and Bcl-2 [29], gaining in turn E-cadherin expression [7], a prerequisite for the correct alignment of the biliary structures, dependent on the "planar cell polarity" (PCP) mechanism. In a recent study, Poncy and colleagues [87] show that Sox9 cooperates with Sox4 to maintain the apical-basal polarity of cholangiocytes, and the correct formation of their primary cilia, to allow the proper ramification of the biliary duct system. A defect in Sox9 has been also related to a delayed biliary differentiation of hepatoblasts lining the asymmetric ducts leading to biliary dysgenesis [87]. Interestingly, biliary dysgenesis is also induced by the inactivation of Notch2 and/or Hes1, although these defects are not sufficient by themselves to completely block the biliary tree formation [40, 57, 67, 112]. These data highlight intensive cross-talk mechanisms among the different morphogens and likely a redundancy of the signaling regulating biliary tree structuring. These data are further corroborated by the studies on Alagille syndrome, a liver disease due to genetic defects in Jag1 and/or Notch2 featuring a hypoplasia of the intrahepatic bile ducts that fail to elongate during fetal development [31, 67]. Another morphogen relevant in liver development is Hedgehog (Hh). Following binding to its receptors, including Patched (Ptc), Hh acts through its downstream effectors, such as Smoothened (Smo), Glioblastoma (Gli), Fused (Fu), Costal2 (Cos2), and others. During the earliest phases of embryonic development, Hh is transiently expressed by the endodermal cells of the ventral foregut and, together

with Gli1, by hepatic progenitor cells (HPCs). Although the function of Hh signaling in bile duct specification and elongation is not completely elucidated, recent data support an important role of Hh in bile duct morphogenesis. Meckel syndrome, a rare autosomal recessive inherited disease, characterized by ductal plate malformation, showed a dysregulation of the Hh signaling [119] likely dependent upon the lack of the primary cilium expressing Ptc, Smo, and Gli [28]. Moreover, Hh signaling is involved in the correct assembly of the liver architecture following partial hepatectomy (PH). In C57BL/6 mice undergoing PH, treatment with cyclopamine, a Smo inhibitor, reduced the proliferation of both hepatocytes and cholangiocytes and inhibited the activation of HPC [82]. Noteworthy, both Notch and Hh signal pathways can modulate Sox9, and their dysregulation led to an altered Sox9 expression phenotypically evolving to liver fibrosis and cancer [54, 78].

TGF-β signaling is another fundamental determinant of biliary morphogenesis since the early stages. High expression of TGF-β is detected around the nascent periportal area, then gradually diminishing through the parenchyma [24]; on the other way, its cognate receptor, TGF-β type II-R, is selectively expressed by the DP cells, albeit transiently. The TGF-β gradient is regulated by several checkpoints provided by HNF6 and OC-2 expressed by DP cells and by miRNA 23b expressed in the parenchymal area [90]. All the members of the TGF-β family (1, 2, and 3) are able to promote the hepatoblast-cholangiocyte shift and to induce the expression of several biliary markers on the DP cells [7, 24]. Furthermore, TGF-β may interact with Notch by modulating the expression of some common targets, such as the transcription factors Hes1 and Hey1, or by upregulating the expression of Jag1 [47].

Another signaling critically involved in several stages of liver development is the Wnt/β-catenin pathway. It governs the proliferation of hepatoblasts and their biliary commitment and also the elongation and the correct tridimensional shaping of the mature biliary structure, through the mechanism of the PCP [39, 77]. The canonical activation of this pathway stimulates the proliferation of hepatoblasts and the expression by DP cells of phenotypic biliary markers, such as K19 and Sox9, in the earliest GWs [76]. In contrast, the noncanonical activation of Wnt/β-catenin signaling stimulates in vitro the formation of duct-like structures with a biliary phenotype in embryonic liver cells challenged with Wnt-3A [52].

As exemplified by TGF-β and Notch, all the abovementioned signaling may cross talk each other and act in concert to allow a correct tridimensional arrangement of the normal biliary tree and to maintain its physical and functional integrity.

Recently, miRNAs have been proposed as novel players of bile duct development, mainly acting as negative regulators. In particular, using a genome-wide approach on *zebra fish*, Hand and colleagues [48] showed that 38 miRNAs changed their levels of expression over the fetal liver development, with miR-30a and miR-30c being those specifically expressed by DP cells. Moreover, Rogler [90] demonstrated that miR-23b expressed in the developing liver by parenchymal but not portal mesenchymal cells is able, in vitro, to repress the cholangiocyte commitment of a fetal liver cell line by interfering with the TGF-β signaling at the level of its small mother against decapentaplegic (SMAD) downstream effectors.